ATCC

Product Sheet

WPE1-NB14 (ATCC® CRL-2850™)

Please read this FIRST

Storage Temp.
liquid nitrogen vapor phase

Biosafety Level
2

Intended Use

This product is intended for research use only. It is not intended for any animal or human therapeutic or diagnostic use.

Complete Growth Medium

The base medium for this cell line is provided by Invitrogen (GIBCO) as part of a kit: Keratinocyte Serum Free Medium (K-SFM), Kit Catalog Number 17005-042. This kit is supplied with each of the two additives required to grow this cell line (bovine pituitary extract (BPE) and human recombinant epidermal growth factor (EGF). To make the complete growth medium, you will need to add the following components to the base medium:

- 0.05 mg/ml BPE - provided with the K-SFM kit
- 5 ng/ml EGF - provided with the K-SFM kit. NOTE: Do not filter complete medium.

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: WPE1-NB14 (ATCC® CRL-2850™)

Description

Organism: *Homo sapiens*, human
Tissue: prostate
Disease: normal
Cell Type: epithelial
Age: 54 years
Gender: male
Morphology: epithelial
Growth Properties: adherent
DNA Profile:
Amelogenin: X
CSF1PO: 13
D13S317: 14
D16S539: 9,11
D5S818: 12,15
D7S820: 10,11
THO1: 8,9.3
TPOX: 8,11
vWA: 18
Cytogenetic Analysis: The depositor reports that at passage 44, a majority of the cells were near diploid, 45-47, X, -Y. Loss of Y chromosome has been observed in prostate cancer.

Batch-Specific Information

Refer to the Certificate of Analysis for batch-specific test results.

SAFETY PRECAUTION

ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.

Unpacking & Storage Instructions

1. Check all containers for leakage or breakage.
2. Remove the frozen cells from the dry ice packaging and immediately place the cells at a temperature below -130°C, preferably in liquid nitrogen vapor, until ready for use.

Handling Procedure for Frozen Cells

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at –70°C. Storage at –70°C will result in loss of viability.

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).
2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions.
3. Transfer the vial contents to a centrifuge tube containing 9.0 mL complete growth medium and spin at approximately 125 x g for 5 to 7 minutes. Discard supernatant.
4. Resuspend the cell pellet with the recommended complete growth medium (see the specific batch information for the culture recommended dilution ratio) and dispense into a 25 cm^2 or a 75 cm^2 culture flask. It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the complete growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6).
5. Incubate the culture at 37°C in a suitable incubator. A 5% CO_2 in air atmosphere is recommended if using the medium described on this product sheet.

Handling Procedure for Flask Cultures

The flask was seeded with cells (see specific batch information), grown, and completely filled with medium at

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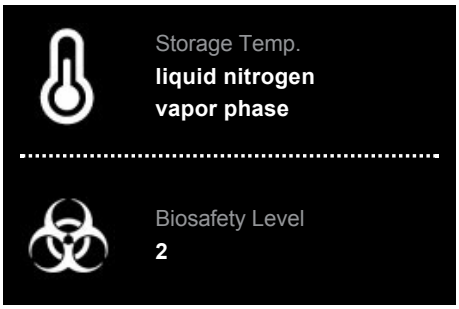

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ATCC to prevent loss of cells during shipping.

1. Upon receipt visually examine the culture for macroscopic evidence of any microbial contamination. Using an inverted microscope (preferably equipped with phase-contrast optics), carefully check for any evidence of microbial contamination. Also check to determine if the majority of cells are still attached to the bottom of the flask; during shipping the cultures are sometimes handled roughly and many of the cells often detach and become suspended in the culture medium (but are still viable).
2. **If the cells are still attached,** aseptically remove all but 5 to 10 mL of the shipping medium. The shipping medium can be saved for reuse. Incubate the cells at 37°C in a 5% CO_2 in air atmosphere until they are ready to be subcultured.
3. **If the cells are not attached,** aseptically remove the entire contents of the flask and centrifuge at 125 x g for 5 to 10 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 mL of this medium and add to 25 cm^2 flask. Incubate at 37°C in a 5% CO_2 in air atmosphere until cells are ready to be subcultured.

Subculturing Procedure

Volumes used in this protocol are for 75 cm^2 flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

Note: Subculture cells before they reach confluence. Do not allow cells to become confluent.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with Ca++/Mg++ free Dulbecco's phosphate-buffered saline (D-PBS).
3. Add 2.0 to 3.0 mL (to a T-25 flask) or 3.0 to 4.0 ml (to a T-75 flask) of 0.05% Trypsin - 0.53 mM EDTA solution, diluted 1:1 with D-PBS, and place flask in a 37°C incubator for 5 to 8 minutes. Observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 10 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach.
4. Add 6.0 to 8.0 mL of 0.1% Soybean Trypsin Inhibitor (or 2% fetal bovine serum in D-PBS), as appropriate, and aspirate cells by gently pipetting.
5. Transfer cell suspension to centrifuge tube and spin at approximately 125 x g for 5 to 7 minutes.
6. Discard supernatant and resuspend cells in fresh serum-free growth medium. Add appropriate aliquots of cell suspension to new culture vessels. An inoculum of 2 x 10^4 to 4 x 10^4 viable cells/cm^2 is recommended.
7. Incubate cultures at 37°C. We recommend that you maintain cultures at a cell concentration between 4 x 10^3 and 8 x 10^4 cells/cm^2.

Note: Cells grown under serum-free or reduced serum conditions may not attach strongly during the 24 hours after subculture and should be disturbed as little as possible during that period.
Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:5 is recommended
Medium Renewal: Every 48 hours
Note: For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 10 in **Culture of Animal Cells, a Manual of Basic Technique** by R. Ian Freshney, 3rd edition, published by Alan R. Liss, N.Y., 1994.

Cryopreservation Medium

Complete growth medium described above supplemented with 15% fetal bovine serum and 10% (v/v) DMSO. Cell culture tested DMSO is available as ATCC® Catalog No. 4-X.

Comments

WPE1-NB14 cells belong to a family of cell lines, referred to as the MNU cell lines, which are all derived from RWPE-1 cells after exposure to MNU. The larger family of cell lines, including RWPE-1 cells with a common lineage, mimics multiple steps in progression from normal epithelium to prostatic intra-epithelial neoplasia, and then to invasive cancer. The MNU cell lines, in order of increasing malignancy are: WPE1-NA22 (ATCC CRL-2849), WPE1-NB14 (ATCC CRL-2850), WPE1-NB11 (ATCC CRL-2851), and WPE1-NB26 (ATCC CRL-2852).
WPE1-NB14 cells show moderate invasive ability in the in vitro Boyden chamber invasion assay Ref.
The colony forming efficiency (CFE) of 1.85% and the invasive ability of WPE1-NB14 cells are both greater than those of WPE1-NA22 cells (ATCC CRL-2849) Ref.
The cells form small tumors after subcutaneous injection. The tumors are a little larger than those formed by WPE1-NA22 cells (ATCC CRL-2849).
The depositor report that the parent RWPE-1 cell line (ATCC CRL-11609) was screened for Hepatitis B and C, and human immunodeficiency viruses, and was found to be negative.

References

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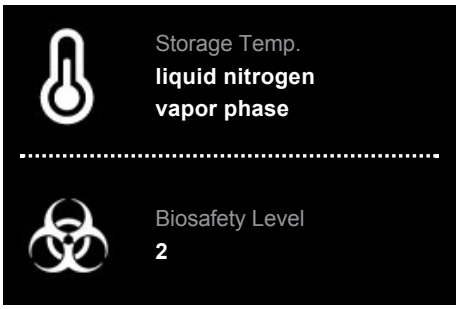

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References and other information relating to this product are available online at www.atcc.org.

Biosafety Level: 2

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the current publication of the *Biosafety in Microbiological and Biomedical Laboratories* from the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health.

ATCC Warranty

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Disclaimers

This product is intended for laboratory research purposes only. It is not intended for use in humans.
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Additional information on this culture is available on the ATCC web site at www.atcc.org.